

US Serial No. 07/565,673
Page 2

LIST OF CLAIMS, SHOWING THE STATUS OF EACH CLAIM

Claims 1-47. (Cancelled)

48. (Currently Amended) A method of obtaining a non-reverting mutant alkalophilic *Bacillus* strain wherein said alkalophilic *Bacillus* strain is *Bacillus* novo species PB92 or the derivative PBT 110, having a reduced level of a wild-type high alkaline serine protease, said method comprising the steps of:

a) transforming an alkalophilic *Bacillus* strain comprising a gene encoding the wild-type alkaline serine protease with a cloning vector comprising DNA encoding a replication function and 5' and 3' flanking non-coding regions of said gene encoding the wild-type high alkaline serine protease but not the coding region of said gene encoding the wild-type high alkaline serine protease gene, wherein a sufficient amount of said 5' and 3' flanking non-coding regions is present to provide for homologous recombination with the indigenous gene encoding the wild-type alkaline serine protease of said alkalophilic *Bacillus* strain whereby transformants having a reduced level of said wild-type alkaline serine protease are obtained;

b) growing said transformants under conditions whereby the replication function encoded by said cloning vector is inactivated; and

and

c) isolating transformants having a reduced level of the wild-type alkaline serine protease.

49. (Cancelled)

50. (Once Amended) A mutant, non-reverting alkalophilic *Bacillus* strain wherein said mutant alkalophilic *Bacillus* strain is *Bacillus* novo species PB92 or the derivative PBT 110, producing a mutant high alkaline serine protease and no detectable level of a wild-type high alkaline serine protease, wherein said mutant, non-reverting alkalophilic *Bacillus* strain is obtained by growing an alkalophilic *Bacillus* strain which is incapable of producing said wild-type high alkaline serine protease transformed with a plasmid expression vector comprising said mutant high alkaline serine protease gene.

GC329Office Action Response 4-26-04

US Serial No. 07/565,673
Page 3

51. (Cancelled)

52. (Cancelled)

53. (Previously Presented) The alkalophilic Bacillus strain of Claim 50 wherein said strain is asporogenic.

54. (Currently Amended) A method for the production of a mutant high alkaline protease, said method comprising the steps of:

a) obtaining an alkalophilic Bacillus host selected from the group consisting of Bacillus novo species PB92 and its derivatives wherein said derivatives retain the characteristics of Bacillus novo species PB92 and said alkalophilic Bacillus host is incapable of producing a wild-type high alkaline serine protease, and comprises a chromosomal deletion of the gene encoding an the wild-type high alkaline serine protease;

b) transforming said alkalophilic Bacillus host with an integration cassette comprising a gene encoding a mutant high alkaline serine protease, wherein said gene encoding the mutant high alkaline serine protease comprises a replacement of at least one amino acid residue in the nucleotide sequence encoding the wild type high alkaline serine protease of Bacillus novo species PB92 or derivative thereof to obtain a non-reverting mutant alkalophilic strain; and

c) growing said mutant alkalophilic Bacillus host under conditions whereby said mutant high alkaline serine protease is expressed.

55. (Previously Presented) The method according to claim 54 wherein the replacement is at an amino acid residue position selected from the group consisting of positions of 160, 216 and 212.